added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.

I. Rejection of Claims 12, 15-16, 21, 23-24, 26-33 and 59 under 35 U.S.C. § 112, first paragraph

Claims 12, 15-16, 21, 23-24, 26-33 and 59 have been rejected under 35 U.S.C. § 112, first paragraph, as not enabling any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with the claims. The Examiner has acknowledged the specification to be enabling for a method of culturing cells from the rodent or human CNS using specific antibodies directed against specific epitopes to obtain a population of neuron-restricted precursor cells with the specific phenotype and morphological properties disclosed in the specification. However, the Examiner suggests that the specification does not reasonably provide enablement for broadly claimed cell culture methods where others are invited to define the required parameters to practice the claimed invention, or for claims directed toward using any antibody directed against unknown epitopes with no known structural characteristics. The Examiner suggests that the name "embryonic cell adhesion

molecule", alone, sets forth no structural characteristics and little functional characterization for generating antibodies specific to a functional "embryonic neural cell adhesion molecule".

Applicants respectfully disagree.

As stated in MPEP § 2164.01, a patent need not teach and preferably omits, what is well known in the art. Also see *In re Buchner*, 929 F.2d 660, 661, 18 USPQ 81, 94 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinefabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984). Structural and functional characteristics of embryonic NCAM were known in the art at the time of filing this patent application. Knowledge of this protein as of the filing date of this patent application is clearly evidenced by the prior art teaching by Blass-Kampmann et al. (J. Neuro. Sci. Res. 1994 37:359-373), already of record in the instant application. In fact, as made clear in the Introduction of Blass-Kampmann et al. embryonic NCAM had been characterized as early as 1983 by Finne et al. (Biochem. Biophys. Res. Commun. 1983 122:482-487) and

Sadoul et al. (Nature (London) 1983 304:347-349). Copies of these references are being provided herewith. Thus, in accordance with MPEP § 2164.01, specific teachings of structural and functional characteristics of embryonic NCAM need not be taught in the instant patent application as they were well known as of the filing date of this application.

The Examiner's concerns relating to antibodies to active equivalents of embryonic neural cell adhesion molecules and to random modifications, mutations, substitutions, additions, deletions or truncations of different embryonic neural cell adhesion-related molecules which no longer recognize the functional embryonic neural cell adhesion molecules required to practice the instant invention are not relevant as such antibodies are not contemplated by the instant invention nor within the scope of the claimed invention. Claims herein are drawn to methods for isolating a pure population of rodent or human CNS neuron-restricted precursor cells. Clearly use of an antibody which no longer recognizes the functional embryonic neural cell adhesion molecule required to practice the claim invention is not within the scope of the claims.

In an earnest effort to advance the prosecution of this case, however, Applicants have amended the claims to clarify that

the antibody used in the method of the present invention specifically recognizes polysialated neural cell adhesion molecule. Support for this amendment can be found in the specification at page 25. Further, Applicants provided evidence of the commercial availability of an antibody to a sialated form of NCAM in the response filed September 20, 2001. This commercially available antibody which recognizes polysialated NCAM is also described in the specification at page 25. The ability of this antibody to distinguish the cell adhesion molecule N-CAM on embryonic axons and growth cones from a 135 kd cell surface glycoprotein, TAG-1, was demonstrated by Dodd et al. (Neuron 1988 1(2):105-16). A copy of this reference is being provided herewith.

This amendment sets forth the structural characteristics of NCAM so that one of skill in the art can routinely generate and/or select antibodies for use in the claimed method. Further, evidence provided in the response filed September 20,2001 relating to commercial availability of an antibody to sialated NCAM also evidences the fact that NCAM is well known in the art.

MPEP §2164.01 is quite clear; as long as the specification discloses at least one method of making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112, is satisfied. The instant specification which contains teachings regarding use of an antibody against polysialated NCAM clearly meets the requirements of enablement for the claims as now amended.

During the last Telephone Interview conducted on April 3, 2002, the Examiner also questioned enablement of claim 28 drawn to a method for producing postmitotic neurons from a pure population of neuron-restricted precursor cells. Applicants respectfully direct the Examiner to Example 12 of the instant application wherein a method for producing postmitotic neurons from a pure population of neuron-restricted precursor cells is disclosed. This disclosure clearly meets the requirements as set forth by MPEP §2164.01 that the specification discloses at least one method of making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim. Accordingly, the enablement requirement of 35 U.S.C. § 112, with respect to claim 28 is also satisfied by the instant specification.

Withdrawal of all rejections under 35 U.S.C. § 112, first paragraph is therefore respectfully requested.

II. Rejection of Claims 21 and 59 under 35 U.S.C. § 112, second paragraph

Claims 21 and 59 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite and incomplete for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner suggests that step (c) of claim 21 and step (b) of claim 59 recite no active method step thereby being ambiguous and constituting incomplete methods. Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have amended these claims to clarify that the purification procedure is selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture. Support for these amendments can be found in the specification and in the claims as originally filed. Please cancel Claim 23 has been cancelled and the dependency of claim 24 has been changed in light of this amendment.

Withdrawal of this rejection is respectfully requested in light of these amendments.

III. Rejection of Claim 28 under 35 U.S.C. § 102(e)

Claim 28 has been rejected under 35 U.S.C. § 102(e) as being anticipated by Boss et al. (U.S. Patent 5,411,883). It is respectfully pointed out, however, that the methods of Boss et al. relate to mixed populations of cells. In contrast, in the instant method, a pure population of neuron-restricted precursor cells is used. Accordingly, in an earnest effort to advance the prosecution of this case and to clarify this distinction, Applicants have amended claim 28 to specify that a pure population of neuronal-restricted precursor cells was used. Support for this amendment can be found throughout the specification, see for example Example 12, and in the claims as originally filed.

Withdrawal of this rejection is respectfully requested in light of this amendment.

IV. Rejection under 35 U.S.C. § 102(b) and 35 U.S.C. § 103

Claims 21, 23 and 26-27 have been rejected under 35 U.S.C.

§102(b) as being anticipated by Blass-Kampmann et al.

Claims 12, 15-16, 24 and 28-33 have also been rejected under 35 U.S.C. § 103(a) as being unpatentable over Blass-Kampmann et al., in view of Boss et al., Weiss et al., Johe et al., Rao et al. and/or Lee et al.

The Examiner suggests that the current claims do not distinguish the instant claims from the method of Blass-Kampmann et al., or in the alternative from Blass-Kampmann et al. in view of Boss et al., Weiss et al., Johe et al., Rao et al., and/or Lee et al. Applicants respectfully disagree.

Differences between the instant claimed method and the method taught by Blass-Kampmann et al. were outlined in detail in the response filed by Applicants on September 11, 2000. As discussed in detail in that response, claims 12, 21, 26 (which depends from claim 12) and 27 (which depends from claim 26) were amended to clarify that FGF containing media was used to support adherent growth, that retinoic acid containing medium promotes differentiation to neurons, and that astrocyte-promoting medium containing FGF and 10% fetal calf serum fails to promote proliferation or differentiation. Neither culture in FGF nor retinoic acid is taught in Blass-Kampmann et al. Instead, in the method taught by Blass-Kampmann, the cells were cultured in

modified Eagle-Dulbecco medium supplemented with 10% FCS or F14 medium supplemented with 10% fetal calf serum, culture conditions now explicitly claimed to neither promote proliferation nor differentiation in the instant invention. Further, Applicants added a step to claim 12 to clarify that the multipotent CNS stem cells were replated on laminin in the absence of chick embryo extract to induce cell differentiation prior to purifying from the differentiating cells the subpopulation of cells expressing embryonic neural cell adhesion molecules. This step is also not taught in the method of Blass-Kampmann et al.

Further, with respect to claims 21 and 27, it is respectfully pointed out that the starting material for the cells is different. The cells of Blass-Kampmann et al. were isolated from fetal brain tissue. In contrast, claims 21 and 27 are drawn to a method and cells produced via a method wherein the cells are isolated from spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube.

In addition, as discussed during a Telephone Interview conducted with the Examiner on February 27, 2002, the population of cells obtained by the method of Blass-Kampmann et al. is not a pure population of neuronal-restricted precursor cells as some

differentiation into glial cells was observed. Accordingly, in an earnest effort to further distinguish the instant invention from the method of Blass-Kampmann et al., Applicants have amended the claims to include a step which removes any cells which differentiate into glial cells. Specifically, the claims have been amended to include a step for removal of A2B5+ cells by specific antibody capture with an antibody that specifically recognizes A2B5 prior to purification of the NEP cells from the resulting supernatant. Support for this amendment can be found in the specification in Table 1 at page 27 wherein a commercially available A2B5 antibody which has been established in the art to recognize oligodendrocytes and precursors is taught and in Example 3, beginning at page 30 wherein removal of A2B5+ cells via specific antibody capture followed by plating of the supernate (see page 31, line 4-5) to purify E-NCAM+ cells is taught.

To anticipate a claim, the reference must teach every element of the claim. See MPEP § 2132. Since Blass-Kampmann et al. does not teach all the steps of the method claimed in the instant application, this reference cannot anticipate the claimed invention. It is therefore respectfully requested that this rejection under 35 U.S.C. § 102 (b) be withdrawn.

With respect to the obviousness rejection under 35 U.S.C. § 103, the secondary references cited in this rejection fail to remedy the deficiencies in the primary reference, Blass-Kampmann et al. as these references also fail to teach or suggest a method for obtaining a pure population of neuronal-restricted precursor cells and any step for removal of A2B5+ cells.

Additional differences between the instant claimed invention and the secondary references cited by the Examiner in the obviousness rejection under 35 U.S.C. § 103 were outlined in detail in the response filed by Applicants on September 11, 2000. As discussed therein, like Blass-Kampmann et al., Boss et al. also teaches different culture medium and method steps to those claimed in the instant invention. For example, at column 7, lines 59-68, Boss et al. teaches that the initial culture medium can be a basal medium supplemented with serum, hormones, growth factors and trace elements. In contrast, as set forth specifically in the claims, culture in 10% fetal calf serum does not promote proliferation or differentiation of the cells of the instant invention. As another example, the differentiating agents taught by Boss et al. at col. 13, lines 43-46, namely sodium butyrate, butyric acid, cyclic adenosine monophosphate derivatives, phosphodiesterase inhibitors, adenylate cyclase

activators and prostaglandins are different to differentiating conditions claimed in the instant invention.

Johe et al. also discloses different culture conditions and steps to those claimed in the instant invention. For example, at col. 7, line 66, through col. 8, line 5, it is taught that differentiation may be directed by adding a second growth factor, namely platelet-derived growth factor, ciliary neurotropic factor, leukemia inhibitory factor or thyroid hormone, iodothyromine.

Weiss et al. disclose methods of differentiating cells by including bFGF. Thus, the method of Weiss et al. is also different from that claimed.

Rao et al. discloses a method for obtaining multipotent stem cells. No method steps or specific conditions for obtaining the neuronal-restricted precursor cells as claimed are either taught or suggested in this Abstract.

Finally, Lee et al. discloses a method for obtaining NT2 cells or NTera 2/cl.D1 cells from a human teratocarcinoma cell line. Accordingly, this reference teaches a completely different starting material to that used in the instant claimed invention. Thus, the teachings of Lee et al. are not predictive for the methods and cells of the instant invention.

To render an invention obvious, the prior art when combined must teach or suggest all the limitations of the claimed invention. See MPEP § 2143. Since the prior art references, either alone or in combination, fail to teach or suggest methods or cells produced by methods with all the steps and culture conditions of the claims as amended, they cannot render obvious these claims. Withdrawal of this rejection under 35 U.S.C. § 103(a) is therefore respectfully requested.

V. Supplemental IDS

In accordance with the Examiner's request, Applicants are providing herewith a Supplemental IDS to enter into the record prior art references discussed in this response.

VI. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The

attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Respectfully submitted,

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Date: **May 3, 2002**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel claim 23.

Please amend the claims as follows:

12. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) isolating a population of rodent or human multipotent CNS stem cells which generate both neurons and glia;

(b) incubating the multipotent CNS stem cells in NEP medium;

(c) replating the multipotent CNS stem cells on laminin in NEP medium in the absence of chick embryo extract to induce cell differentiation;

(d) removing A2B5+ cells from the differentiating cells via specific antibody capture with an antibody that specifically recognizes A2B5;

(e) purifying from the ~~differentiating cells~~ supernatant following step (d) a subpopulation of cells expressing embryonic neural cell adhesion molecules via a

procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, wherein said procedure uses an embryonic neural cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule (NCAM); and

~~(e)~~ (f) incubating the purified subpopulation of cells in a FGF-containing medium configured for supporting adherent growth thereof to obtain an isolated, purified population of rodent or human CNS neuron-restricted precursor cells, wherein said neuron-restricted precursor cells differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

21. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) removing a sample of spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube ~~but prior to differentiation of glial and neuronal cells in the neural tube~~;

(b) dissociating cells comprising the sample of spinal

cord tissue removed from the embryo;

(c) removing A2B5+ cells from the dissociated cells via specific antibody capture with an antibody that specifically recognizes A2B5;

(d) purifying from the ~~dissociated cells via an embryonic neural cell adhesion molecule antibody~~ supernatant following step (c) a subpopulation expressing embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, using an embryonic neural cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule;

~~(d)~~ (e) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

~~(e)~~ (f) incubating the plated cells at a temperature and in an atmosphere conducive to growth to obtain an isolated, pure population of neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF for adherent growth, differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing

medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

24. (amended) The method of claim ~~23~~ 21 wherein said procedure is specific antibody capture.

28. (amended) A method of producing postmitotic neurons from a pure population of neuron-restricted precursor cells comprising:

(a) culturing a pure population of neuron-restricted precursor cells which require FGF and differentiate into CNS neuronal cells but not into CNS glial cells in proliferating conditions; and

(b) changing the culture conditions of the neuron-restricted precursor cells from proliferating conditions to differentiating conditions, thereby causing the neuron-restricted precursor cells to differentiate into postmitotic neurons.

59. (amended) A method of isolating a pure population of mouse or human CNS neuron-restricted precursor cells comprising the steps of:

(a) providing a sample of mouse or human embryonic stem cells;

(b) removing A2B5+ cells from the sample of mouse or human embryonic stem cells via specific antibody capture with an

antibody that specifically recognizes A2B5;

(c) purifying from the ~~mouse or human embryonic stem cells via an embryonic neural cell adhesion molecule antibody~~ supernatant from step (b) a subpopulation expressing embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, using an embryonic cell adhesion molecule antibody specifically recognizes polysialated neural cell adhesion molecule;

~~(c)~~ (d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

~~(d)~~ (e) incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF and differentiate into CNS neuronal cells but not into CNS glial cells.